The Male Mating Strategy of the Bee *Nomia nevadensis* (Hymenoptera: Halictidae): Leg Structure and Mate Guarding

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Abstract.—Males of the bee *Nomia nevadensis bakeri* Cockerell congregate in large numbers within the previous generation's nesting area and mate with emerging virgin females. Competition among males, much of which occurs below ground before the female emerges, and the fact that females are receptive to only one male upon emergence (confirmed experimentally) favors males that: a) search for evidence (possibly odor) that a female is about to emerge at a specific location; b) rendezvous with a female before she emerges from the ground; and c) upon finding a female, use behavioral and morphological means to prevent takeover by other males. Evidence is presented that males use flattened expansions of their hind tibia to grip females firmly. The activities of males in the emergence area makes them conspicuous targets for predation by robberflies (Asilidae).

Females of many species of solitary ground-nesting bees and wasps nest in dense aggregations. Thus, adult virgin females emerging the following generation may provide mate-searching males with a clumped source of receptive females. If these females mate only once, or several times over long periods in their lives, there is a selective advantage to traits that aid males to find mates as close as possible to the time at which the females become receptive (Thornhill and Alcock, 1983). It may even be beneficial for males to attempt to reach a female before she emerges from the ground (e.g., Alcock *et al.,* 1976; O'Neill and Evans, 1983; Schöne and Tengö, 1981). However, in many situations, the high density of conspecific males makes it difficult, even for those finding females early, to complete copulation without interference from competitors. A variety of mechanisms have evolved to minimize such interference including removing females from the vicinity of competitors or guarding the female in a manner that prevents takeovers. In some insects, guarding ability is enhanced by the presence of morphological structures that help the male secure a hold upon the female (Thornhill and Alcock, 1983).

This paper reports on a study of the mating strategy of males of the bee *Nomia nevadensis bakeri* Cockerell. Females of this and other species of *Nomia* construct multicellular nests in extremely dense aggregations (Cross and Bohart, 1960; Kerfoot, 1964; Johansen *et al.*, 1978), such that large numbers of males and females emerge the following year within a restricted area. We studied a dense natural aggregation of *N. nevadensis bakeri* in the summers of 1984 and 1985. Here we present information on the mating strategy of males, the behavioral and

morphological adaptations that may help them avoid interference from competing males, and the predation risk undertaken by mate-searching males. We also conducted experiments to determine whether females are receptive to more than one male upon emergence.

MATERIALS AND METHODS

Nomia nevadensis bakeri was studied on five days between 28 July and 11 August, 1984 and on five days between 24 July and 2 August, 1985. The study site was located beside a dirt road approximately 9 km northeast of Roggen, Weld County, Colorado, U.S.A. The soil in the emergence area was sandy, with a surface crust about 0.5 to 1.0 cm thick. The area from which female bees emerged measured approximately 2×10 m in 1984 and 2×5 m in 1985, and contained sparse vegetation, primarily sunflowers (*Helianthus sp.*) and scurfpea (*Psoralea lanceolata* Pursh).

This aggregation was observed for a total of 24.3 hours over the ten days of study. Focal observations were made on interactions of males with females and conspecific males at emergence holes. Occurrences of all observed matings and predations upon males were recorded and mating pairs were collected and preserved in order to determine the size of mating males and emerging females. An estimate of the body size distribution of males in the population was made from three sweep net samples taken in the emergence area on two days in 1984. Head widths of both males and females were determined to the nearest 0.1 mm with a VWR Scientific Products micrometer accurate to 0.05 mm. In 1985, 53 males were marked on the thorax with dots of enamel paint to facilitate later identification.

We preserved mating pairs in natural positions so that they could be returned to the laboratory to examine the posturing and positioning of the males' legs. In the field, pairs were immersed and stored in liquid nitrogen (boiling point: $-195.8°C$); they were examined under a dissecting microscope immediately upon removal from the liquid. This technique was possible because, once a male grasped a female, we could usually transfer them to the liquid nitrogen without causing them to separate. Liquid nitrogen is commonly used for rapid freezing of biological specimens (Dawes, 1979).

We conducted an experiment to determine if females were receptive to the mating attempts of more than one male upon emergence. For each manipulation, we recorded whether the female mated with the male to which she was presented. In one experimental group (29 females), each was allowed to complete copulation with the male that had found her upon emergence. Following this, each female was presented to a second male. To determine if manipulating females in this way affected their receptivity, each of 15 females in a control group was separated, prior to copulation, from the male that was mounted upon her as she emerged; each was then presented to a second male. As a second experimental group, 12 of the females from the control group were presented to a third male after the second interaction was complete. The protocol for this experiment is outlined in Table 1.

RESULTS

Searching and Mating Behavior. Males were active in the emergence area from about 900 to 1300 hours when soil surface temperatures ranged between about 25° and 45°C. At the peak of activity, hundreds of males swarmed over the emergence

Table 1. Experimental protocol and results of mating experiments. Females used in #3 were those used originally in #2.

	Female Response to Manipulation	
Protocol	Female Refused to Mate	Female Copulated
1) Copulation completed; female then paired with another male.	29	0
2) First copulation interrupted before coupling; female then paired with second male.	0	15
3) Mated female from #2 paired with third male.	12	0

area within 10 cm of the surface. Each male flew in an irregular pattern, occasionally landing to investigate holes, 0.5 to 1.0 cm in diameter, in the surface of the sand. The lack of a tumulus or depression around these holes indicates that they were emergence holes, rather than active nest entrances (Kerfoot, 1964). No nesting females were seen in the area during the course of the study. After landing near a hole, a male usually stood facing the entrance or entered it to remain underground for from several seconds to over a minute. As many as four males were observed within in a single hole at a given time with others standing near the entrance, facing the hole, when a female was about to emerge.

Initial pairing of males and females always occurred below the surface within the tunnels before the female had emerged from the ground. Each female emerged from a hole with a male mounted upon her, although they had not coupled genitalia at this time. During the study, we saw males digging at the surface only three times. Apparently, waiting males entered holes after the female broke through to the surface. However, this is an inference, since we never observed the exact moment when the emergence hole was opened. Usually within 60 seconds of emerging with the female, if other males were not in contact with the pair, the mounted male moved back along the female's body, probed with his genitalia, and coupled. After coupling genitalia, the male's abdomen began to pulsate rhythmically and he often released the female from his leg grasp. During this entire period, a receptive female remained quiescent and did not attempt to break free from the male. This copulatory phase lasted from 4 to 43 seconds (mean = 19.2; SD = 8.8; N = 42). The male then broke genitalic contact and left or, more often (90% of 42 cases), moved forward on the female's body to his original position and remained for 2 to 72 seconds (mean = 22.2; SD = 16.8; N = 38). Following this post-copulatory phase, the male broke contact and the female immediately flew away from the emergence area. Males often reentered the swarm following copulation. On one day in 1985, six males that mated were marked after they had copulated. Five of these males were resighted in the swarm within 5 to 29 minutes. Males were also observed within the emergence area up to seven days after they were marked.

All mating pairs were collected within several cm of an emergence hole. Males were never observed flying and carrying a female away from the emergence area. A total of 271 mating pairs were seen during the course of the study. Most of the matings (N = 246; mean = 18.1/hour of observation) were observed in the first year of the study. During the second year, activity was much lower probably due to heavy rains before and during the seasonal period of activity, and only 25 matings were observed (mean = 2.3/hour of observation).

Emerging females released a strong, "sweet" odor. If a female (mated or unmated) was held in a pair of forceps or within the end of an insect net 5 to 10 cm above the surface of the ground within several minutes of her emergence, males always approached upwind in a rapid zig-zag flight and landed upon her, often causing a struggle among males for the female. This procedure never failed to attract males to recently emerged females (alive or dead; N = 66) or to males that had recently been in contact with a live female. The entire sequence of copulation could be initiated in this way if the female was a virgin (see next section).

On five occasions we observed males investigating (i.e. walking in tight circles and antennating the soil surface) localized areas (several cm^2), but not in the vicinity of a hole. All five times we scraped away the surface soil, once to a depth of 5 cm, and discovered a female who was releasing the strong scent. Apparently, males, possibly by orienting to the "sweet" odor, can detect females that have not yet reached the surface. By doing so, they could wait for the females at the exact point of emergence. The presence of the surface crust may act to delay the female just below the surface and increase the chance that she will be discovered by a male.

Female Receptivity in the Emergence Area. We were able to demonstrate that females will mate with only one male between the time that they emerge and the time they leave the emergence area. Females that had already copulated always refused to mate with other males (Table 1, #1 & #3), although the latter made vigorous mating attempts. On the other hand, females that were separated before copulation from the first male mounted upon them were always receptive (Table 1, #2). The latter data indicate that our technique was not responsible for non-receptivity of the females used in the above experiments.

When a female was not receptive to mating attempts she had means of preventing copulation, even though the male often had a secure hold upon her. The form of these interactions, which sometimes lasted longer than 5 minutes, was distinctly different from those that resulted in successful copulation. To refuse a mating attempt. a female curled her abdomen forward until her genitalic region nearly touched her head. This prevented the male from making genitalic contact. She also attempted to pry herself loose from the male by pushing backwards and upwards at him with her hind legs and by beating her wings if they were free. Typically, she walked forward during these attempts. None of these behaviors were seen in interactions that resulted in successful copulation. Eventually, unreceptive females were able to break free and leave the emergence area without further interference from males. Outside of our experiments, we observed one instance of a female refusing to mate with a male in the emergence area.

Interactions among Males. There is potential for competition among males both below and above ground. We have noted that a number of males may enter an emergence hole. By peering into the emergence holes, we often observed what were apparently intense struggles for females, though we were unable to record the

duration of such interactions or determine whether they involved interference or scramble competition.

Above ground, after the female emerged with a male mounted upon her back, one to four males were frequently in contact with the pair during the pre-copulatory phase, struggling to gain access to the female. The males were mounted either dorsally on the first male's back or ventrally beneath the female. Generally, the posture and behavior, particularly of the dorsally mounted males, was similar to that of a male mounted upon a female. Some of these interactions may simply have been mistaken attempts by males to mount females. The pervasive odor of the female could have been responsible for a failure of males to discriminate between the sexes. We also observed twelve cases of isolated "homosexual" pairs (i.e. males mounted upon other males). Odor has been implicated as the trigger for inappropriate sexual mounts in other insect species in both natural (Tomkins et al., 1980; Tengö, 1979) and experimental (Shimron and Hefetz, 1985) situations.

We detected no size-biased mating success among males. Males sampled from copulating pairs (mean head width = 3.01 mm; S.D. = 0.07; N = 89) were not significantly different in size from males in sweep net samples taken in the emergence area (mean = 3.01 mm; S.D. = 0.08; N = 252; t-test, t_{339} = 0). On the other hand, females taken in the emergence area (mean = 3.19; S.D. = 0.09; N = 66) were not only significantly larger than copulating males on average (t-test, t_{153} = 14.2; p < 0.001), but in each mating pair were either larger than (95%) or equal to (5%) the male in size (N = 43). There were no females present among the 252 bees taken in the sweep samples, giving further confirmation that they do not remain in the emergence area after mating (although they must return later to nest).

Morphological Modifications for Mate Guarding. The structure and exact placement of the hind legs may combine to prevent the mounted male from being supplanted by others during the pre-copulatory phase. The male's head was just behind that of the female's, with his front and middle legs usually held over her wings, preventing them from moving. His hind legs were wrapped around her abdomen just posterior to her petiole, usually between the first and second gastral segments. Above ground at least, males were never supplanted by others, when positioned in this manner. In addition, when an unreceptive female was presented to a male, it was difficult for her to break free, although the male could not induce her to mate. In hundreds of observed interactions, a pair was disrupted during the post-copulatory phase only twice, and during the pre-copulatory phase only once. In the latter case, the male did not have his legs properly situated under the female's abdomen; she mated with the usurping male.

By immersing pairs that were in the pre-copulatory position into a Dewar flask containing liquid nitrogen, we were able to return five pairs from the field intact, for closer examination. Only the right side of each pair was examined, since manipulation caused the pair to separate slightly.

A structure on the males' hindlegs that may help them grasp a female was evident: the tibia is expanded distally to form a pair of flattened triangular flanges (Fig. 1) that lie flat against the female's abdomen, pointing anteriorly, when a male has his legs wrapped around a female. The positioning of the flanges on the males' tibiae suggests that they locked onto the female by sliding the larger one beneath the posterior edge of a sclerite beneath her abdomen (Fig. 2). On one female the larger flange was almost completely inserted beneath the ventral posterior edge of gastral tergum I
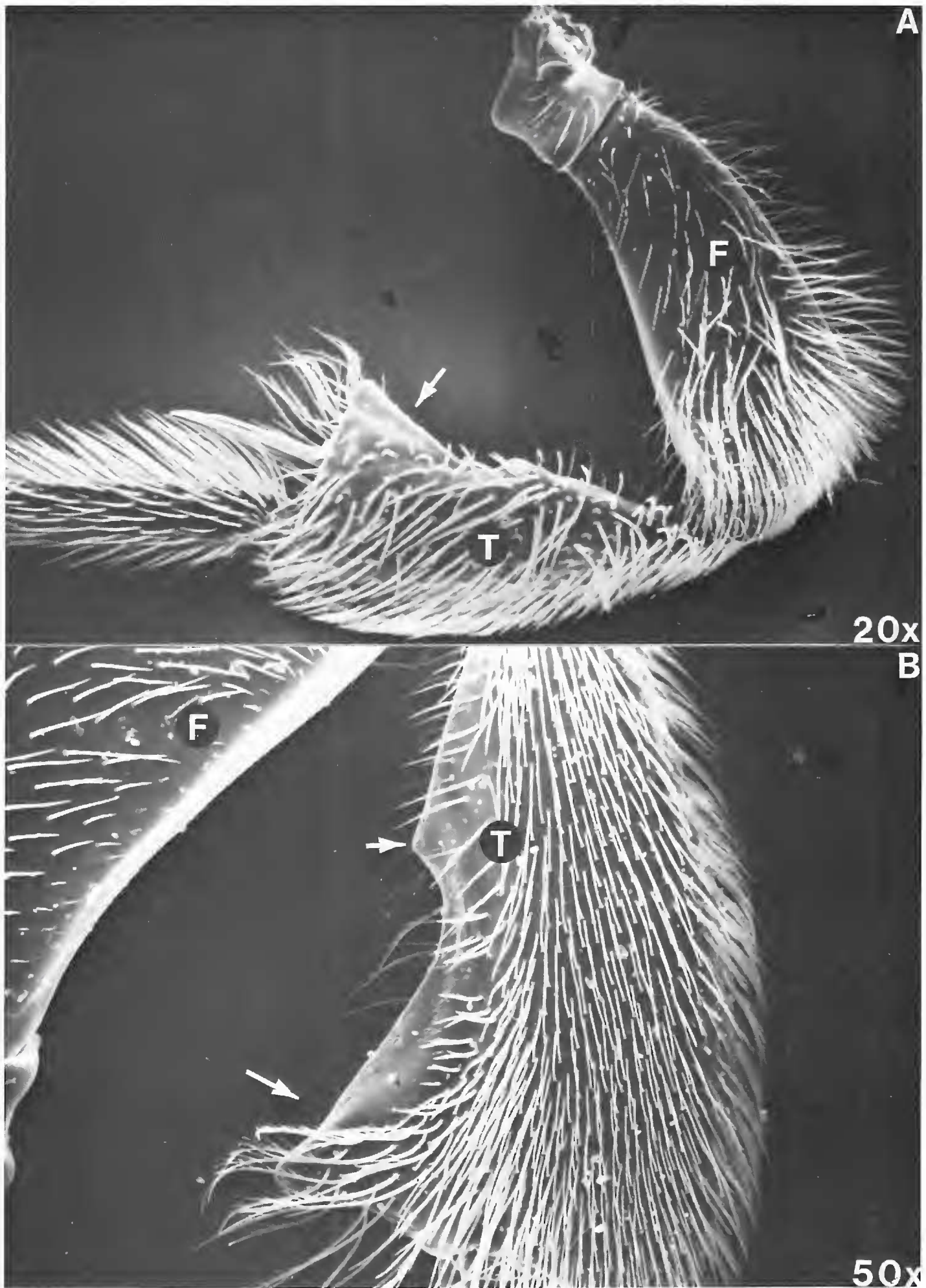
Figure 1. A) Scanning electron micrograph of the left hindleg of male of *N. nevadensis bakeri*, (20X; side of tibia shown is that which lies against the female when the male is mounted in the pre-copulatory posture); B) Close-up view (50X) of right hind tibia (opposite aspect from A) of male showing expansions (arrows); F = femur; T = tibia.
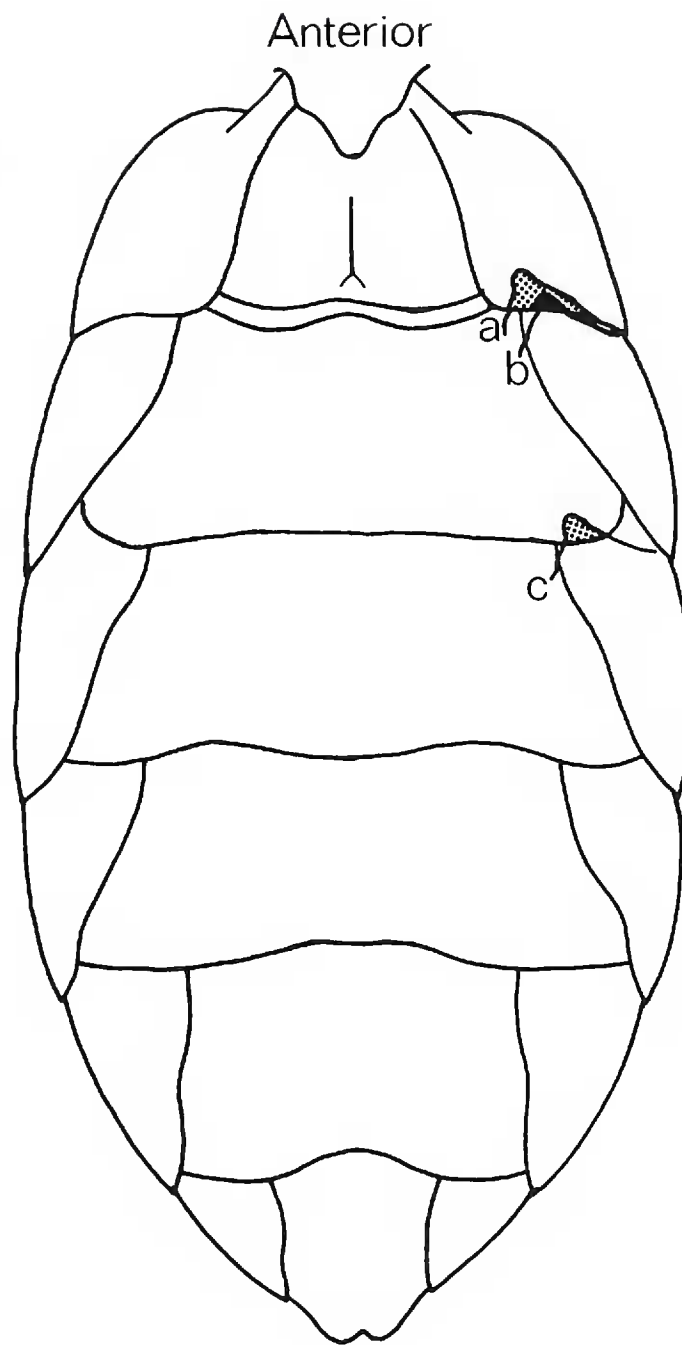
Anterior

Figure 2. Schematic representation of the venter of the abdomen of a female *N. nevadensis bakeri*, showing where the large flange of the male hind tibia was positioned on five pairs examined. One each found in position (a) and (c) (stippled area denotes portion inserted under female's sclerite); three found in position (b) (black area denotes portion inserted).

where the latter curls beneath the abdomen (Fig. 2,a). The tip of the flange was so inserted when examined on three others (Fig. 2,b), although it may have pulled out slightly in the process of the examination. On the fifth female, approximately half of the flange was inserted beneath the posterior edge of sternum II (Fig. 2,c). The tarsal segments of the males' legs were lying back along the medial line of the female's venter.

Predation upon Males. Males were sometimes preyed upon by robberflies of the species *Diogmites angustipennis* Loew. These flies, which reach up to 22 mm in length (Lavigne and Holland, 1969), were apparently attracted by the conspicuous movements of male bees flying about the emergence area. Twenty-three successful

predations were observed, the majority (21) of which occurred on the one day in which the flies were most abundant.

DISCUSSION

Females of *Nomia nevadensis bakeri* in this population were receptive upon emergence, mated with only one male while in the emergence area, were apparently detectable prior to emergence, possibly because of the odor they emit, and emerged within an area containing many conspecific males. It is also probable that some degree of protandry occurs in this population, as it does in other species of *Nomia* (Kerfoot, 1964; Cross and Bohart, 1960; Johansen *et al.,* 1978). The combination of these factors creates a competitive situation favoring males that reach and mate with an *emerging* female before conspecific males do. To accomplish this, males in this population: 1) search for evidence of females about to emerge, 2) attempt to rendezvous with females before they emerge from the ground, and 3) upon finding a female, use behavioral and morphological means to prevent other males from usurping their position. Males were rarely seen digging where a female was about to emerge. This contrasts with other species of Hymenoptera with similar mating systems (e.g., Alcock *et al.,* 1976; O'Neill and Evans, 1983; Schöne and Tengö, 1981), where males invest much time and energy digging for pre-emergent females.

Male insects competing for females within a crowded emergence area use a variety of means to prevent interference form conspecific males (Thornhill and Alcock, 1983). Males of some species of bees and wasps avoid takeover by competitors by carrying the female away in flight from the emergence area before mating with her (Alcock *et al.,* 1976; O'Neill and Evans, 1983). However, it would have been difficult for males of *N. nevadensis* in this population to do this, since they were never larger than the female and would probably have had difficulty carrying her in flight. The male is usually larger than the female in insects in which the male carries the female during courtship or mating (O'Neill, 1985). In contrast to other species of Hymenoptera in which larger size has been shown to aid males in their attempts to obtain matings (e.g., Alcock *et al.,* 1976; O'Neill, 1983a,b; O'Neill and Evans, 1983; Severinghaus *et al.,* 1981), no such effect was detected in our analysis. Males of *N. nevadensis* show much less range in size than species of digger wasps (O'Neill, 1985) and bees (Alcock, *et al.,* 1976) for which a size advantage among competing conspecifics has been shown, although the reason for this is unclear. We can speculate on one factor selecting *against* large size in this species: males that are too large may be unable to maneuver for position on a female within an emergence hole. However, this hypothesis would be difficult to test.

Rather than leave the emergence area with the female, males of *N. nevadensis* remained. Those males grasping females with the aid of modified leg structures were highly successful at maintaining contact with a female and completing copulation. It could also be hypothesized that these leg structures function to subdue the female so that mating can take place, much as male scorpionflies use their notal organ to force copulation upon females (Thornhill, 1980). However, this seems unlikely for several reasons. First, virgin females were generally quiescent during mating attempts, so need not have been subdued. Second, as demonstrated in the mating experiments, previously mated females were capable of refusing to copulate and could break free from the males. Therefore, it seems more likely that the behavior and leg structure of

males have evolved as traits that prevent interference from conspecific males, thus assuring sole access to a receptive female.

Males of a variety of insect species utilize leg modifications to maintain a grip upon females during courtship and mating. Males of other species of the subgenus to which *N. nevadensis* belongs (*Epinomia*) also have flattened expansions of their hind tibia (Cross, 1958). Males of some species of the subgenus *Acunomia* possess even larger expansions of the hind tibia that are not flattened like they are in *N. nevadensis* and are sometimes larger than the main part of the tibia itself (Ribble, 1965); this indicates that these tibial modifications may be utilized in a slightly different manner than they are in *N. nevadensis,* if indeed they are used at all during courtship and mating in these other species. The hind femora are also enlarged in some species of *Nomia.* We have not been able to find reference to the potential use of these leg structures in other species of *Nomia.* Males of the melittid bee *Meganomia binghami* Cockerell use enlargements of the hindlegs to assist in maintaining a grip upon females in the presence of up to ten conspecific males (Rozen, 1977; Stage, 1971). Male bees of some species of the genus *Agapostemon* (Halictidae) have similar modifications of the hindlegs, although their function is unknown (Roberts, 1969, 1972). The unmodified structure of the legs of males of most species of bees contrasts markedly with that of males of *Nomia, Agapostemon,* and *Meganomia* and with females of most non-parasitic species (Stephen *et al.,* 1969). Given the morphological specialization associated with foraging evident in the hind legs of female bees (e.g., Roberts and Vallespir, 1978), leg structure in bees appears to have maintained a high degree of evolutionary plasticity.

Some species of beetles (Crowson, 1981) and flies (Spieth, 1952) also have leg structures that provide a firm grasp upon mates. For example, in the fly *Sepsis cynipsea* (L.), the front legs of a male are structured so that they act as a clamp around the female's wing bases. In this species, mating occurs at cattle droppings where up to 500 males may be present (Parker, 1972). Males of a variety of insects possess modifications of wings, genitalia, jaws, and antennae that are used to grip females (Thornhill and Alcock, 1983; Rothschild and Hinton, 1968).

Along with our observations on *Nomia,* the comparative information suggests that sexual selection has promoted the independent evolution of a variety of morphological structures that enhance the mating success of individual males through an ability to grasp females securely in the presence of competitors. The ability to manipulate interactions among male and female *Nomia* and, potentially, the leg structure of males, may provide a good system for experimental and comparative studies of the function and efficiency of mate guarding. Our brief study also leaves open questions concerning the below ground competition for females, the function of the male's remaining with the female for a short time after insemination, and cues used by males to locate pre-emergent females.

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